

FIG. 1

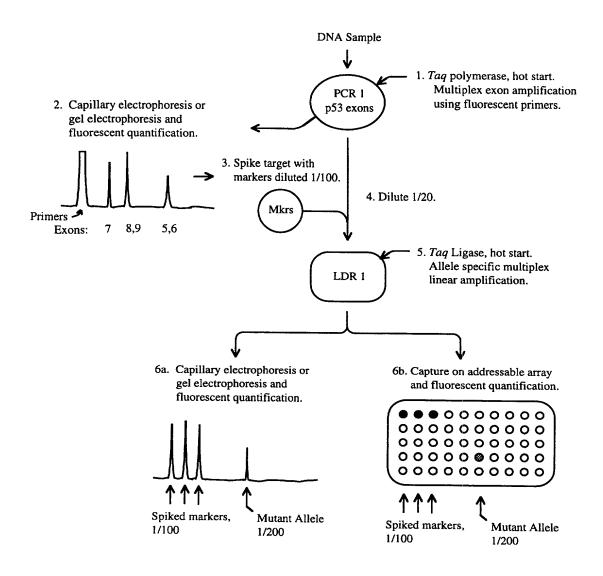


FIG. 2

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A or G

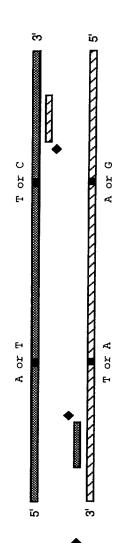
T or A

Z3 Z4

Z1 Z2

## PCR/ LDR

 PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.

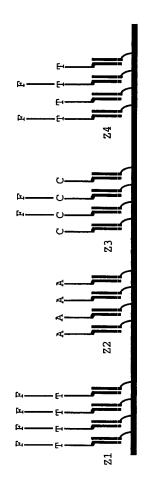


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2. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele specific oligonucleotides ligate to

thermostable ligase. 
Allele specific
oligonucleotides ligate to
common oligonucleotides
only when there is
perfect complementarity
at the junction.

3. Capture fluorescent products on addressable array and quantify each allele.

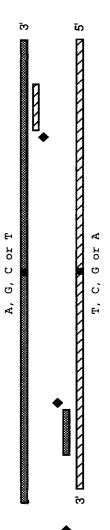


Homozygous: T allele only.

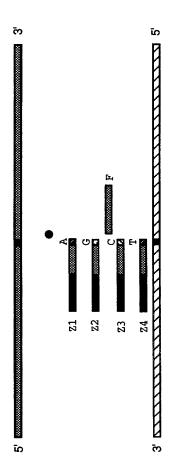
Heterozygous: C and T alleles.

## PCR/LDR

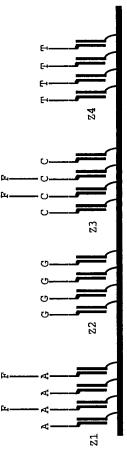
 PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.



2. Perform LDR using allele-specific LDR primers and thermostable ligase. Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



C, G or A



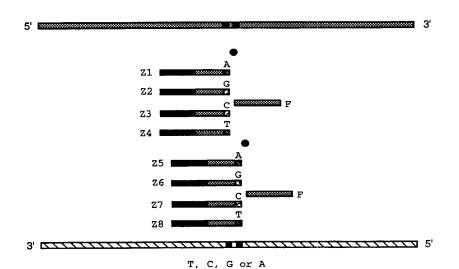
Heterozygous: A and C alleles.

#### PCR/LDR: Nearby alleles

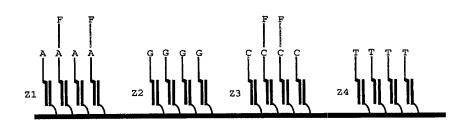
- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.
- A, G, C or T

  5'

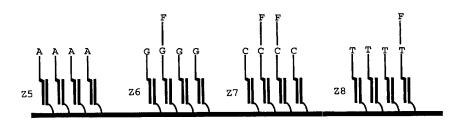
  T, C, G or A
- 2. Perform LDR using allele-specific LDR primers and thermostable ligase. Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A and C alleles.



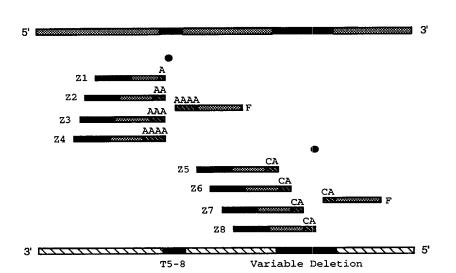
Heterozygous: G,C, and T alleles.

#### PCR/ LDR: Insertions and Deletions

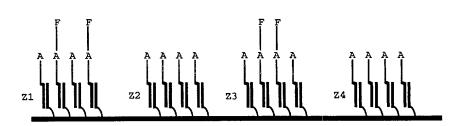
- PCR amplify region(s) containing mutations using primers, dNTPs and Tag polymerase.
- A5-8 Variable Deletion in (CA)n

  5'

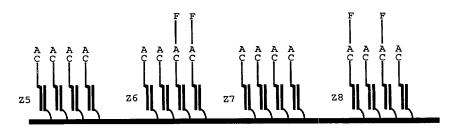
  T5-8 Variable Deletion in (GT)n
- 2. Perform LDR using allele-specific LDR primers and thermostable ligase. Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A5 and A7 alleles.



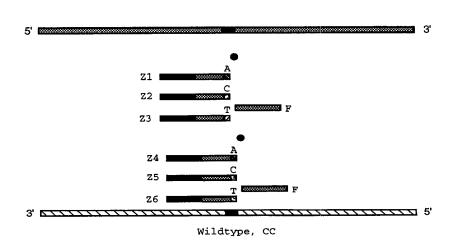
Heterozygous: (CA)5 and (CA)3 alleles.

#### PCR/ LDR: Adjacent alleles, cancer detection

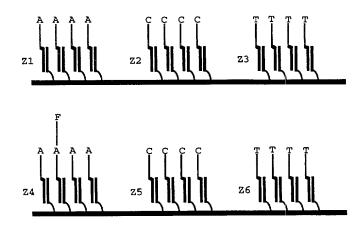
- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.
- Wildtype, GG

  5'

  Wildtype, CC
- 2. Perform LDR using allele-specific LDR primers and thermostable ligase. Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



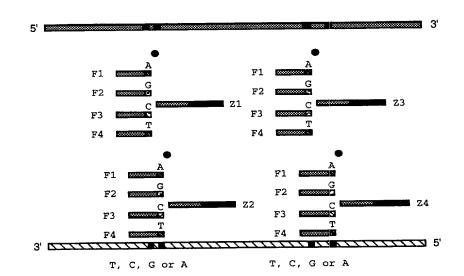
 Capture fluorescent products on addressable array and quantify each allele.



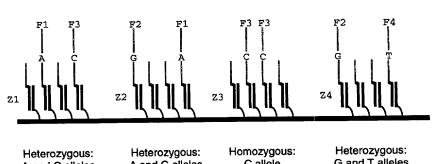
Gly to Asp mutation

#### PCR/LDR: Nearby alleles

- 1. PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.◆
- A, G, C or T A, G, C or T T, C, G or A T, C, G or A
- 2. Perform LDR using allele-specific LDR primers and thermostable ligase. Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



A and C alleles.

A and G alleles.

C allele.

G and T alleles.

#### PCR/ LDR: Adjacent and Nearby alleles

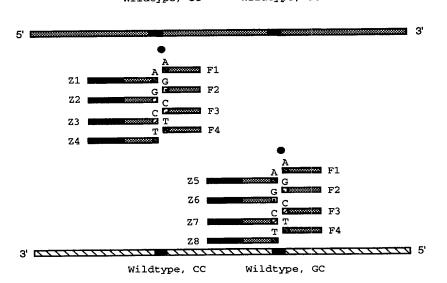
- PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.
- Wildtype, GG Wildtype, CG

  5'

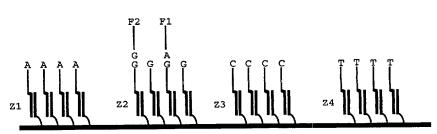
  Wildtype, CC Wildtype, GC

2. Perform LDR using allele-specific LDR primers and thermostable ligase. 

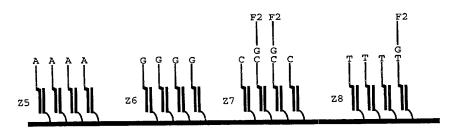
Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



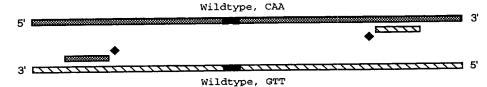
Heterozygous: Gly and Glu alleles.



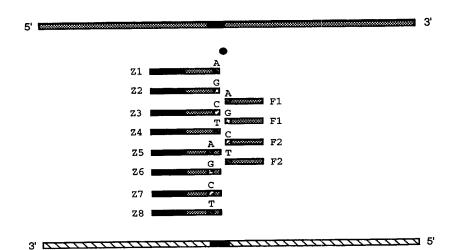
Heterozygous: Arg and Trp alleles.

#### PCR/ LDR: All alleles of a single codon

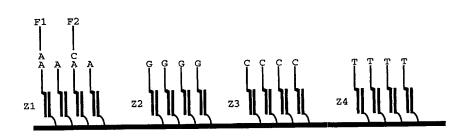
 PCR amplify region(s) containing mutations using primers, dNTPs and Taq polymerase.



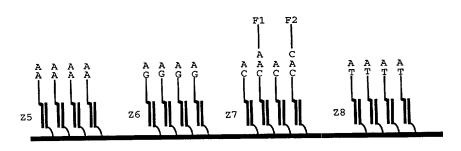
2. Perform LDR using allele-specific LDR primers and thermostable ligase. ■ Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



 Capture fluorescent products on addressable array and quantify each allele.



Wildtype, GTT



Heterozygous: Gin and His alleles.

FIG. 10

$$X$$
  $X^*$   $Y$ -PEG  $Y$ 

W = protecting group, e.g. Boc, Fmoc

Z = activating group, e.g. imidazole (Im), p-nitrophenol (OPnp), hydroxysuccinimide (OSu), pentafluorophenol (OPfp)

PEG = oligo or poly(ethylene glycol), backbone  $(CH_2CH_2O)_n$  n = 6 to 200 (can also be grown by anionic polymerization with  $\sqrt{\phantom{a}}$ )

WSC = water soluble carbodiimide

#### Functional group transformations/activation (as needed), $X \rightarrow X^*$ , $Y \rightarrow Y^*$

$$\begin{array}{l} -\mathrm{OH} \longrightarrow -\mathrm{O(CH_2)_nCO_2H} \quad n=1,2 \\ -\mathrm{OH} \longrightarrow -\mathrm{O(C=O)NHCH_2CO_2H} \\ -\mathrm{OH} \longrightarrow -\mathrm{O(C=O)CH_2NH_2} \\ -\mathrm{OH} \longrightarrow -\mathrm{O(C=O)Im} \\ -\mathrm{OH} \longrightarrow -\mathrm{O(C=S)SCH_2(C=O)NH_2} \\ -\mathrm{CO_2H} \longrightarrow -(\mathrm{C=O)NH(CH_2)_nNH_2} \quad n=2,6 \\ -\mathrm{CO_2H} \longrightarrow -(\mathrm{C=O)Z} \\ -\mathrm{NH_2} \longrightarrow -\mathrm{NH(C=O)(CH_2)_nCO_2H} \quad n=2,3 \end{array}$$

#### Covalent linkage, X\* + Y\*

FIG. 12C

Trp<sub>n</sub>—Probe + HOCH<sub>2</sub>—QCH<sub>2</sub>)<sub>4</sub> C—Support

CH<sub>3</sub>O

dilute acid

Trp<sub>p</sub>—Probe

$$CH_3O$$
 $CH_3O$ 
 $C$ 

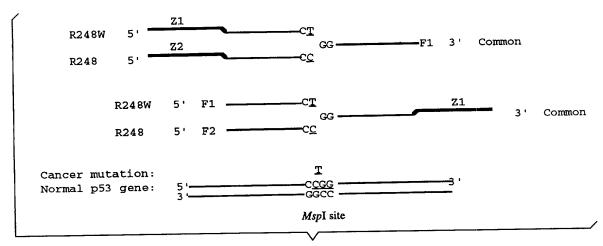
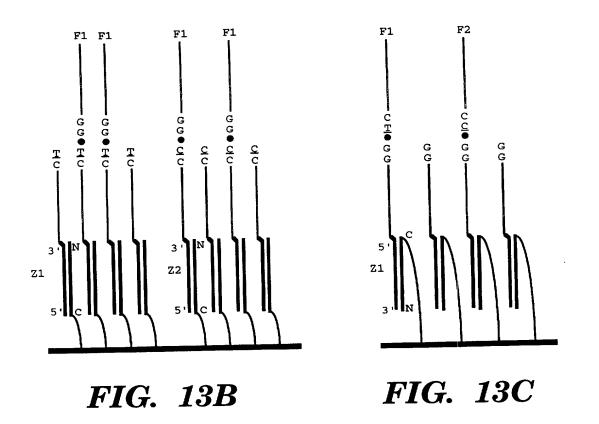
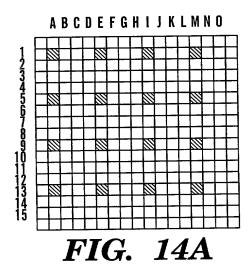
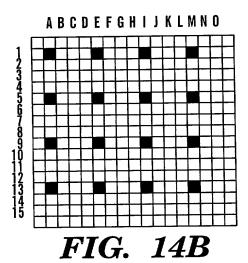
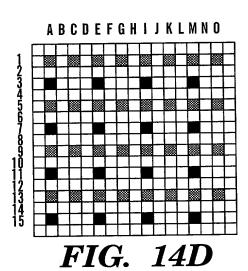


FIG. 13A









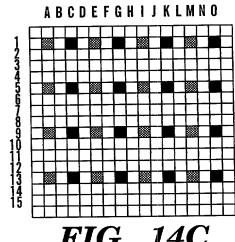


FIG. 14C

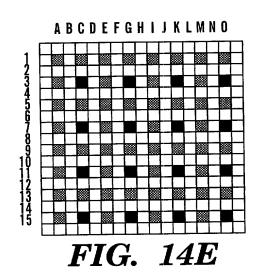


FIG. 15A

1st addition of unique 24mers.

## FIG. 15B

2nd addition of unique 24mers.

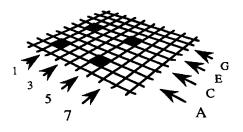


FIG. 15C
3rd addition of unique 24mers.

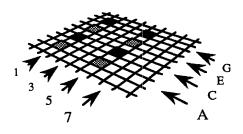
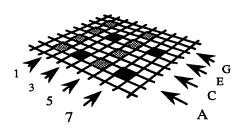
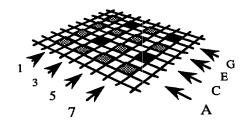


FIG. 15D
4th addition of unique 24mers.





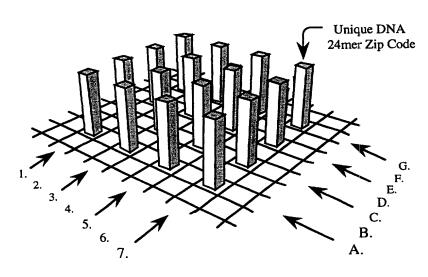


FIG. 15E

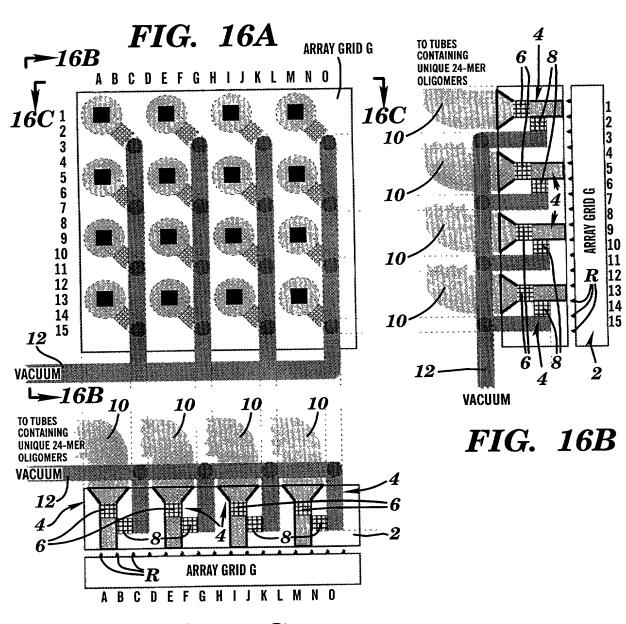


FIG. 16C

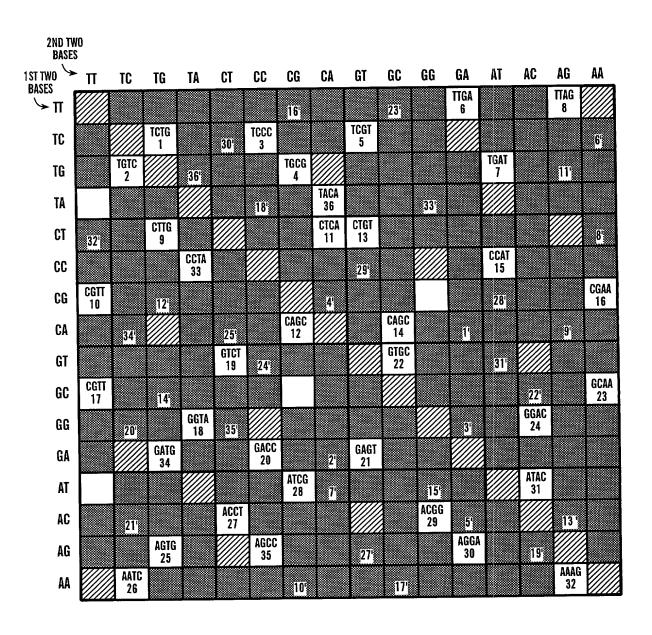


FIG. 17

1st Tetramer addition
(columns)

1	2	3		4	١	5			
1	2	3	3	4		5			
1	2	3	,	4		5			
1	2	3	3	4		5			
1	2	3	3	4		5			

FIG. 18A

## 4th Tetramer addition (rows)

2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5
4	4	4	4	4

FIG. 18D

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2nd Tetramer addition (rows)

	6	6	6	6	6
	5	5	5	5	5
	4	4	4	4	4
	3	3	3	3	3
Ī	2	2	2	2	2

FIG. 18B

## 5th Tetramer addition (columns)

6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4

FIG. 18E

## 3rd Tetramer addition (columns)

		ı		_	1	_	í
3	4		5	6		1	
3	4		5	6		1	
3	4		5	6		1	
3	4		5	6		1	
3	4		5	6		1	

FIG. 18C

## 6th Tetramer addition (rows)

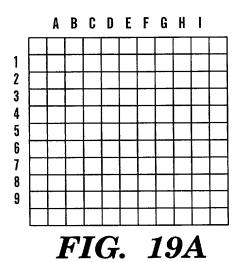
3	3	3	3	3
2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5

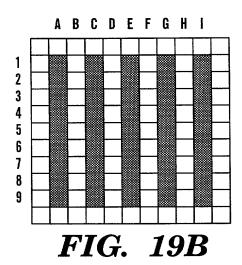
FIG. 18F

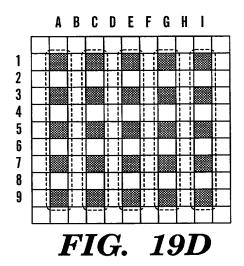
### Addressable array with full length PNA 24mers

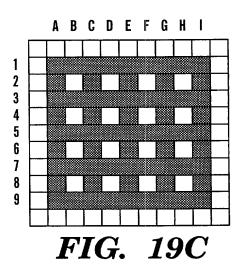
_		_						. 1		
	1-6-3-2-6-3		2-6-4-2-1-3		3-6-5-2-2-3		4-6-6-2-3-3		5-6-1-2-4-3	
	1-5-3-1-6-2		2-5-4-1-1-2		3-5-5-1-2-2		4-5-6-1-3-2		5-5-1-1-4-2	
	1-4-3-6-6-1		2-4-4-6-1-1		3-4-5-6-2-1		4-4-6-6-3-1		5-4-1-6-4-1	
	1-3-3-5-6-6		2-3-4-5-1-6	5	3-3-5-5-2-6		4-3-6-5-3-6	5	5-3-1-5-4-6	
	1-2-3-4-6-5		2-2-4-4-1-5	5	3-2-5-4-2-5	5	4-2-6-4-3-	5	5-2-1-4-4-5	5

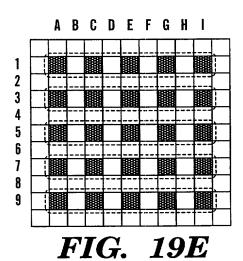
FIG. 18G

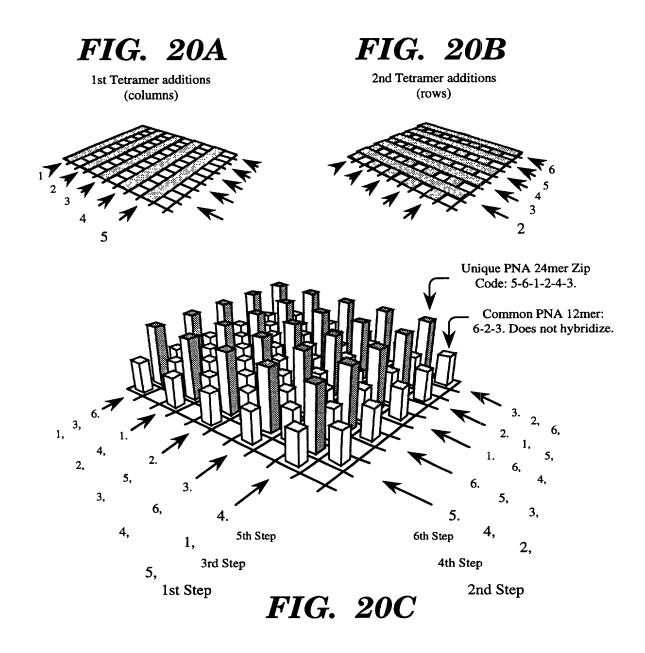


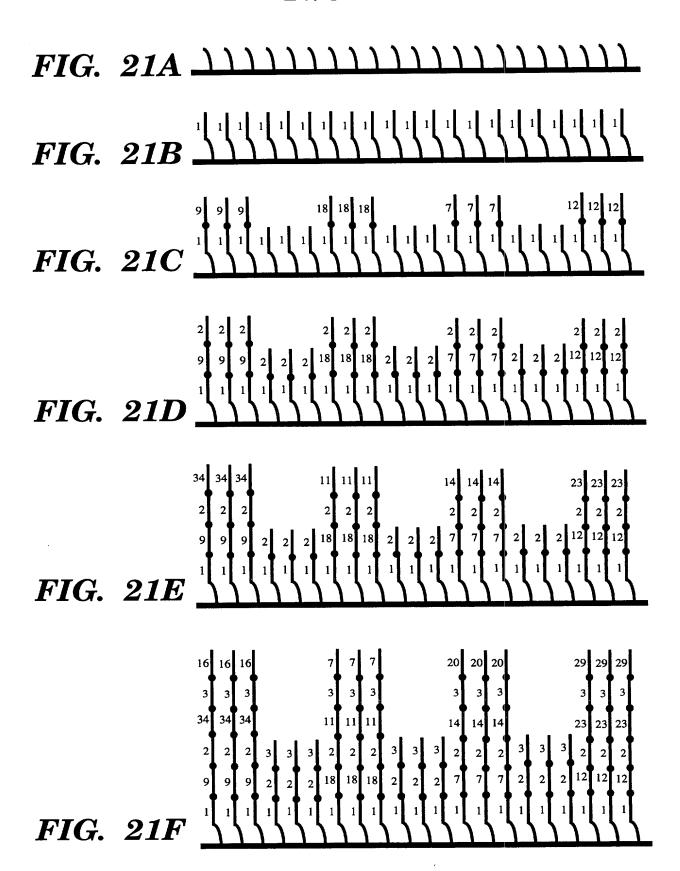


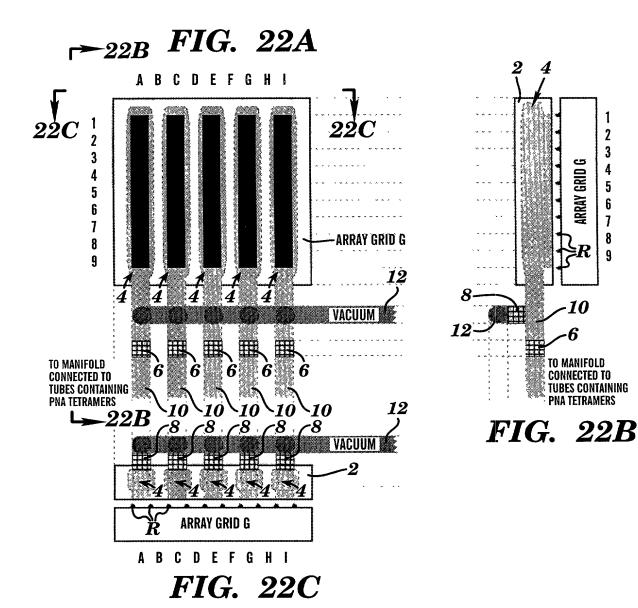


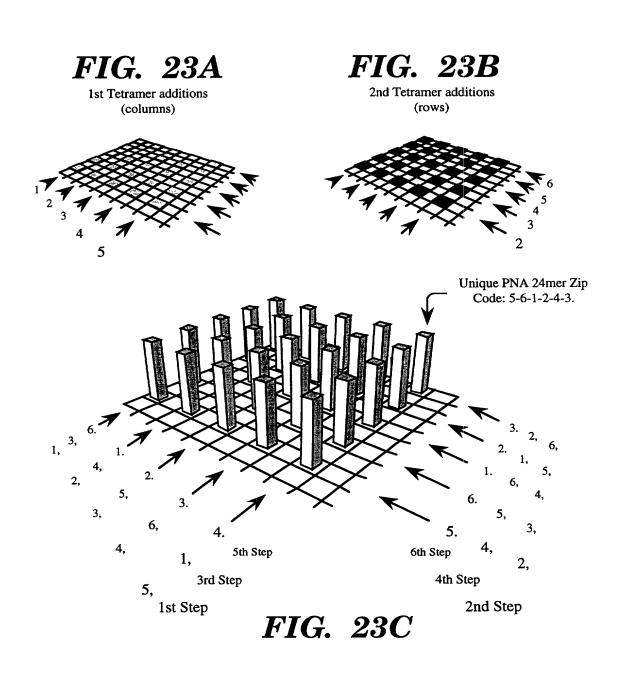


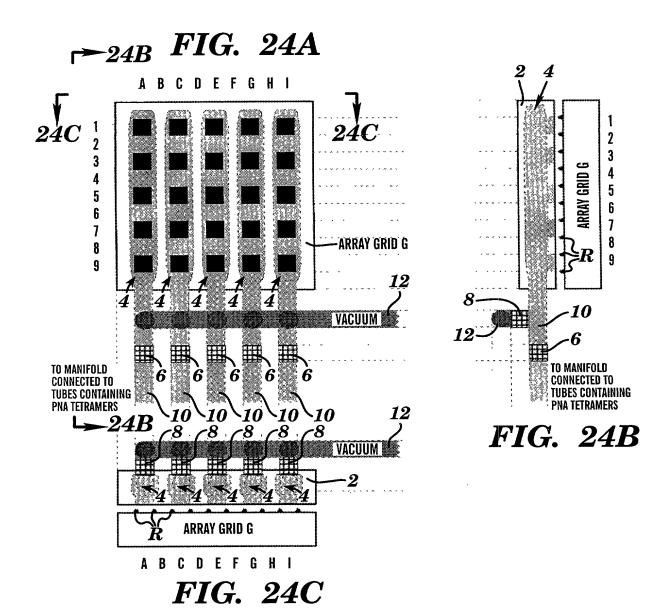


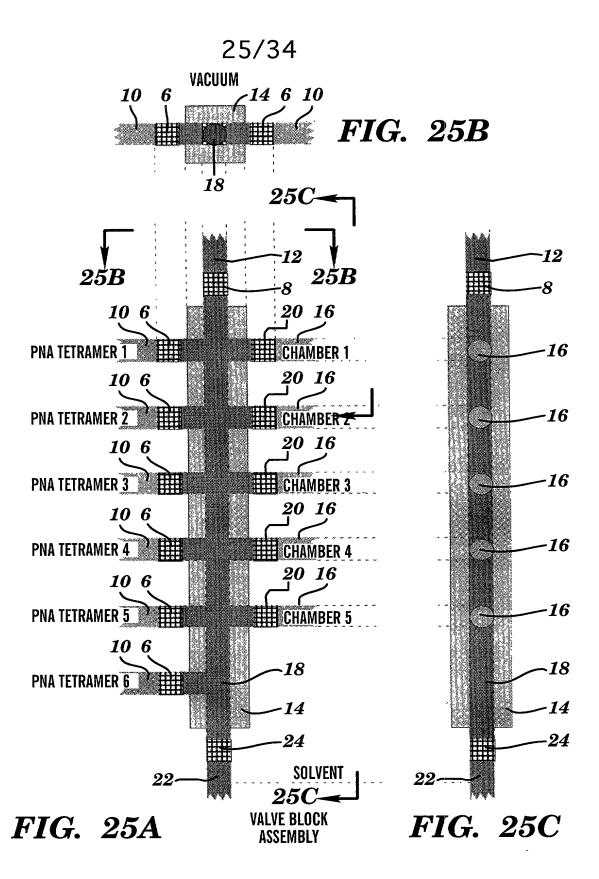




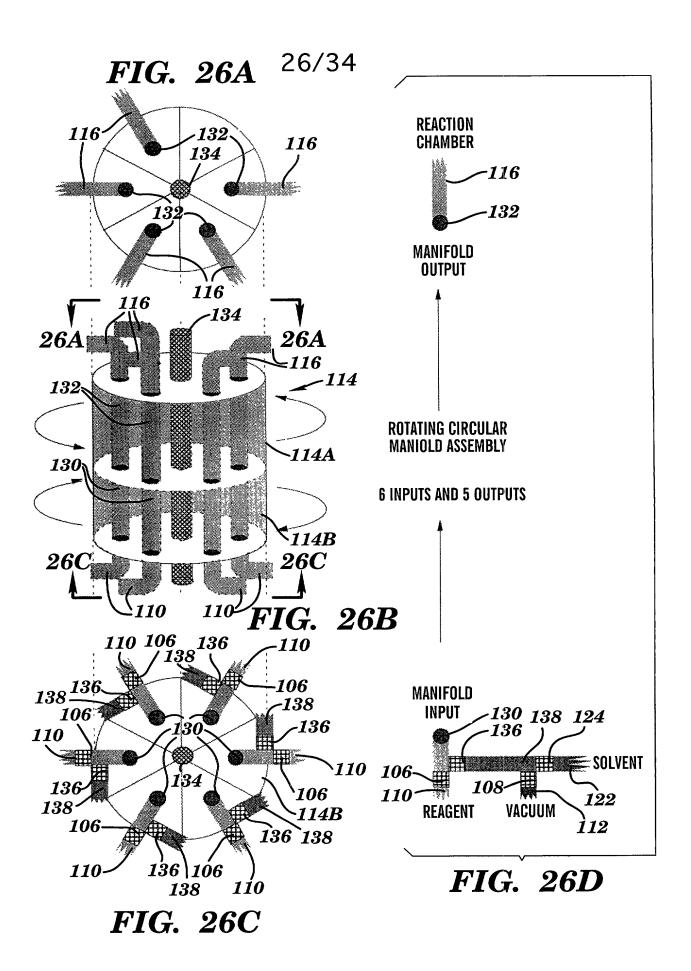








**6 INPUTS AND 5 OUTPUTS** 



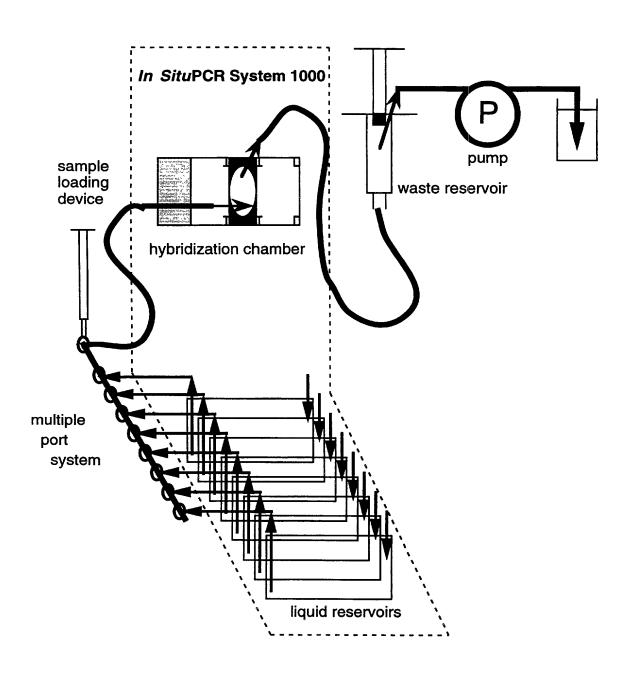


FIG. 27

-COOH; PROBE 12

-COOH; PROBE 14

-NH2; PROBE 12

-NH2; PROBE 14

2% EGDMA

2% HDDMA

4% EGDMA

1 2

FIG. 30

IV953556 L19551

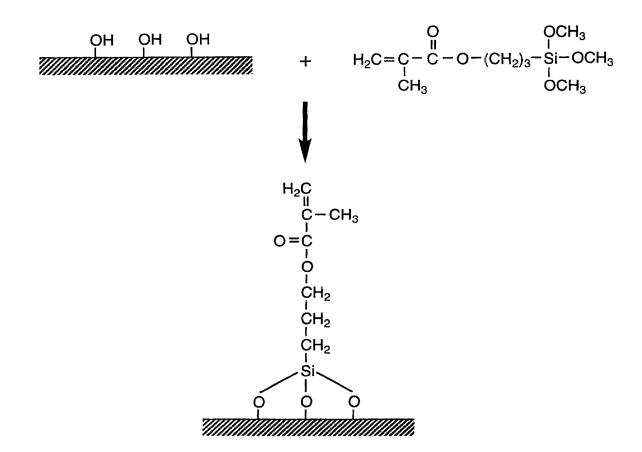


FIG. 31

FIG. 32

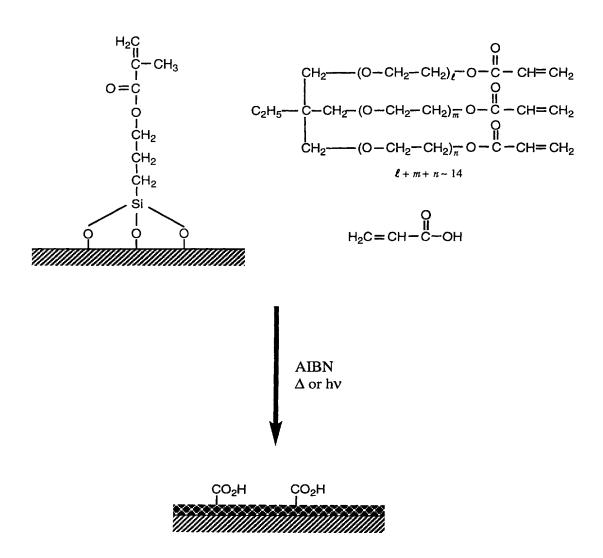


FIG. 33

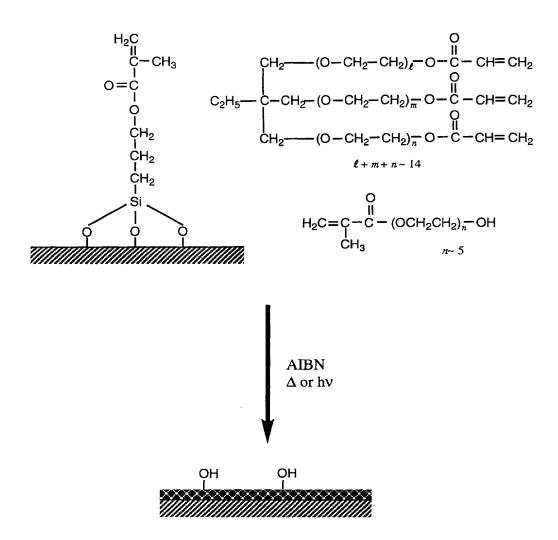


FIG. 34